

**Table 1**  
Details of the new synovitis scoring system.

| Score | Synovitis Score Parameters |                  |  |   |
|-------|----------------------------|------------------|--|---|
|       | Hoffa Synovitis            | Joint Effusion   | Synovial Thickening (measured when JE > 0) | Synovial Proliferation (measured when JE > 0)             |
| 0     | Normal                     | < 2mm            | Normal, almost invisible                   | (NA)  |
| 1     | Mild hyperintensity        | ≥ 2mm and < 5mm  | < 2mm                                      | Smooth  |
| 2     | Moderate hyperintensity    | ≥ 5mm and < 10mm | ≥ 2mm and < 4mm                            | Mild irregularity, bands, small bodies                    |
| 3     | Severe hyperintensity      | ≥ 10mm           | ≥ 4mm                                      | Irregular villonodular proliferation, bands, large bodies |

**Table 2**  
Prevalence of each score (# of patients).

| Score | Prevalence      |                |                     |                        |
|-------|-----------------|----------------|---------------------|------------------------|
|       | Hoffa Synovitis | Joint Effusion | Synovial Thickening | Synovial Proliferation |
| 0     | 3               | 18             | 2                   | (NA)                   |
| 1     | 22              | 14             | 24                  | 4                      |
| 2     | 18              | 11             | 4                   | 22                     |
| 3     | 5               | 5              | 0                   | 4                      |

**455**  
**EFFECTS OF TISSUE CALCIFICATION DURING OSTEOARTHRITIS ON EXTRACELLULAR MATRIX AND CARTILAGE STIFFNESS**

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**Purpose:** In a previous study we showed that osteoarthritic changes of human articular cartilage are accompanied by tissue calcification, which correlates with disease severity. During this pathological process, chondrocytes undergo phenotypic changes from a resting state towards hypertrophy, recapitulating endochondral ossification. Here, we investigated, if the calcified osteoarthritic tissue exhibits altered biomechanical properties compared to the non calcified osteoarthritic samples, which might contribute to disease severity.

**Methods:** Formalin-fixed, paraffin-embedded sections of human articular knee cartilage from eight different donors with varying grades of osteoarthritis (ranging from OARSI grade 0–6) and varying grades of calcification (0–2% of total articular cartilage) were analyzed via histology and immunohistochemistry. Total calcification was measured via digital-contact radiography (DCR) to detect macro-calcification, whereas micro-calcification was evaluated via von-Kossa staining. Alcian blue (pH1.0)/PAS staining was performed to visualize the negatively charged proteoglycan content and antibodies against certain subspecies of chondroitin sulfate disaccharide units ( $\Delta$ COS,  $\Delta$ C4S and  $\Delta$ C6S, respectively) were used to further analyze alterations of the glycosaminoglycan chains. In addition, unfixed cartilage samples were examined for their compressive stiffness using atomic force microscopy and the results were correlated with disease state and grade of calcification.

**Results:** With increasing disease severity, but without signs of calcification, the negative charge of the tissue decreases, as indicated by a decreased Alcian blue staining. Intriguingly, we found an enhanced Alcian blue staining in samples, if osteoarthritic changes were accompanied by calcification of the matrix. Therefore, we investigated the sulfation pattern of proteoglycans. In all samples, no staining for  $\Delta$ COS was detectable, regardless of disease state and grade of calcification.

Articular cartilage without calcification and no signs of osteoarthritis showed a homogenous staining for  $\Delta$ C4S within the superficial layer and the upper part of the middle zone, but no staining in the deeper parts of the tissue, whereas almost no staining for  $\Delta$ C6S could be observed. Upon increasing disease severity (OARSI  $\leq 2$ ) but without calcification,  $\Delta$ C4S staining showed no striking differences compared to OARSI grade 0 tissue, but a slight increase in  $\Delta$ C6S staining was visible. These samples showed a decrease in mean compressive stiffness (down to 60 kPa) compared to non calcified OARSI grade 0 tissue (100 kPa). In osteoarthritic samples (OARSI  $\geq 2$ ) accompanied by increasing tissue calcification, the staining for  $\Delta$ C4S increased and also extended into the deeper cartilage zones, together with a distinct increase in  $\Delta$ C6S staining. Interestingly, the compressive stiffness in these samples was much higher (up to 190 kPa) compared to the non-calcified osteoarthritic cartilage samples or the control cartilage.

**Conclusions:** We could confirm in this study, that due to enhanced catabolic processes and the resulting matrix degradation during osteoarthritis, the compressive stiffness of osteoarthritic tissue decreases with increasing disease severity. Interestingly, osteoarthritic tissue with increasing calcification exhibits increased compressive stiffness and increased extracellular matrix sulfation. If the increase in compressive stiffness is a direct consequence of calcification or an indirect, because of enhanced sulfation of the tissue, has to be further investigated.

**456****A REFERENCE DATABASE OF CARTILAGE T2 VALUES IN KNEES WITHOUT CARTILAGE DEGENERATION, AND DIFFERENCES IN CARTILAGE T2 BY DEMOGRAPHICS: DATA FROM THE OSTEOARTHRITIS INITIATIVE**

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**Purpose:** The purpose of this study was 1) to establish a gender- and BMI-specific reference database of cartilage T2 values, and 2) to assess the associations between cartilage T2 values and gender, BMI and age in knees without radiographic Osteoarthritis (OA) (Kellgren-Lawrence (KL) 0/1) and without morphological evidence of degeneration on 3T MRI.

**Methods:** 481 subjects between the ages of 45–65 years with KL 0/1 at baseline in the study knee were selected from the Osteoarthritis Initiative database. Baseline morphologic cartilage MRI readings whole-organ magnetic resonance imaging scores (WORMS) and T2 measurements were performed in the medial femur, lateral femur, medial tibia, lateral tibia, and patella compartments. We analyzed joint compartments with WORMS cartilage scores of 0–1 and applied a logarithmic transformation to obtain the 5th–95th percentile values for cartilage T2. Linear regression models were used to assess the relationship between (1) cartilage T2 and age (adjusted for gender, BMI, WOMAC pain score, and clinical site), (2) cartilage T2 and gender (adjusted for age, BMI, WOMAC pain score, and clinical site), and (3) cartilage T2 and BMI (adjusted for age, gender, WOMAC pain score, and clinical site) after log transformation.

**Results:** Within each compartment, there was substantial T2 variability among subjects (~10ms range between the 5th and 95th percentiles) (Table 1). Overall, a positive association between cartilage T2 and both age and BMI was observed (Table 1, Fig. 1). The association between age and T2 was most pronounced in the medial femur (1.40% increase in median T2/10 years,  $p=0.050$ ), and the patella (3.27% increase in median T2/10 years,  $p=0.009$ ) (Fig. 1). A positive association between BMI and cartilage T2 was evident in all cartilage compartments and was most pronounced in the lateral tibia (5.33% increase in median T2/5 kg/m<sup>2</sup> increase in BMI,  $p<0.0001$ ). Obese subjects (BMI=30–45 kg/m<sup>2</sup>) had significantly higher ( $p<0.02$ ) mean T2 values than subjects with a “normal” BMI (BMI=18–24.9 kg/m<sup>2</sup>) and subjects that were “overweight” (BMI=25–29.9 kg/m<sup>2</sup>) in the lateral tibia, medial femur, and medial tibia compartments. When investigating the association between gender and cartilage T2, we observed that females had slightly higher cartilage T2 values than males in a majority of compartments; however, the differences were only significant in the medial femur ( $p<0.0001$ ) (Table 1).

**Conclusions:** This study established the first reference database of cartilage T2 values in a large sample of morphologically normal cartilage plates in knees without radiographic OA. While higher BMI was most significantly associated with higher knee cartilage T2, we also observed

<sup>1</sup> The first two authors contributed equally to this work.

a positive, yet weak, association between age and T2. In addition, females demonstrated slightly elevated cartilage T2 compared to males. Overall, this database can serve as a reference for future studies performed using the same techniques, and will enable comparisons to studies that focus on cohorts with expected abnormal T2 values.

**Table 1:** Reference values for cartilage T2 based on gender and BMI groups in the medial femur.

|                | N   | 5%   | 25%  | 50%  | 75%  | 95%  |
|----------------|-----|------|------|------|------|------|
| Females        | 225 | 34.1 | 36.3 | 38.0 | 39.8 | 42.4 |
| Males          | 198 | 33.5 | 35.5 | 37.0 | 38.5 | 40.8 |
| Normal BMI     | 184 | 33.8 | 35.9 | 37.5 | 39.1 | 41.6 |
| Overweight BMI | 145 | 33.7 | 35.8 | 37.3 | 38.8 | 41.2 |
| Obese BMI      | 94  | 33.7 | 36.2 | 38.0 | 39.9 | 42.9 |

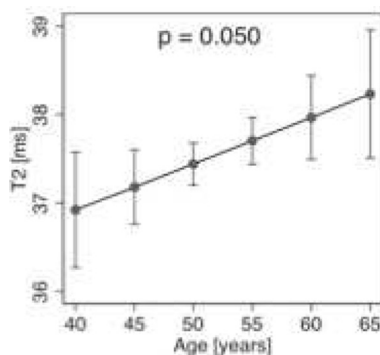


Figure 1: The association between adjusted mean cartilage T2 and age in the medial femur.

#### 457 ESTABLISHMENT OF SURGICAL DESTABILIZATION MODEL OF MOUSE ANKLE OSTEOARTHRITIS

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**Purpose:** Incidence rate of ankle osteoarthritis (OA) is much lower than that of knee OA. Previous studies have described the reasons for the difference in susceptibility to cartilage destruction of the two joints from various study fields including anatomy, biomechanics, biochemistry and molecular biology. However, in vivo experiments to confirm the association of these factors with the susceptibility have not been performed due to lack of a proper animal model of ankle OA.

To address this issue, we aimed to develop a surgical destabilization model of ankle OA in mice. In the present study, we created two different destabilization models; the medial model and the mediolateral model, and compared the histological changes and scores of ankle joints in the two models.

**Methods:** 8-week-old C57BL/6 mice were used. Anatomy of tendons and ligaments of the ankle was shown in Figure 1. For the medial model, tibialis posterior tendon (TP), deltoid ligament (DL) and medial ankle capsule (MAC) were resected in 13 mice (7 males and 6 females). For the mediolateral model, TP, DL, MAC, anterior talofibular ligament (ATFL), calcaneofibular ligament (CFL), peroneus longus tendon (PL), peroneus brevis tendon (PB) and lateral ankle capsule (LAC) were resected in 12 mice (6 males and 6 females). Mice were sacrificed 8 weeks after the surgery, and the ankle joints were fixed in 4% paraformaldehyde for 24 hours. Whole joints were decalcified in EDTA for 5 days on a shaker. Joints were then embedded in paraffin and 4 µm frontal sections were taken through the entire joint. Slides were stained with Safranin-O and Fast green. Each ankle yielded 5–10 slides for scoring using a semi-quantitative scoring system recommended by Glasson in 2010 for the mouse knee OA model. Histological scoring was made of the tibiotalar joints (sum of tibial and talar surface scores) and the subtalar joints (sum of talar and calcaneal surface scores). Results were expressed as the mean ± standard error of the mean (S.E.M.) of the maximum score.

**Results:** Representative sections of the normal, the medial model and the mediolateral model mice were shown in Figure 2. The tibiotalar joint cartilage was degenerated in both the medial and mediolateral models. In both models, OA lesion increased from center to medial part of the tibiotalar joint. Subtalar joint cartilage degeneration was more severe in the mediolateral model than in the medial model. The summed tibiotalar joint scores were  $6.8 \pm 0.9$  in the medial model, and  $6.9 \pm 1.0$  in the mediolateral model, while the summed subtalar joint scores were  $2.6 \pm 1.3$  in the medial model, and  $4.8 \pm 1.0$  in the mediolateral model with significant difference between two groups. When we sequentially sacrificed the medial model mice at 2, 4, 6 and 12 weeks after the surgery, the OA lesion gradually progressed throughout the period, while the cartilage destruction was completed by 6–8 weeks after the surgery.

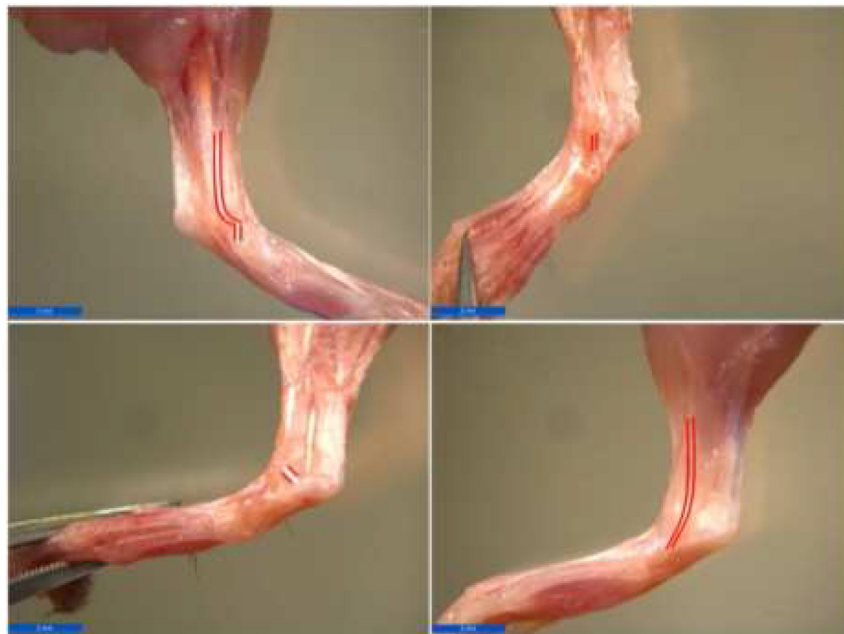


Figure 1. The left ankle anatomy of a mouse. Red double line shows tendons and ligaments for resection in our destabilization models. Tibialis posterior tendon (TP) and deltoid ligament (DL) in the medial side (upper left panel). Anterior talofibular ligament (ATFL, upper right panel), calcaneofibular ligament (CFL, below left panel), peroneus longus tendon and peroneus brevis tendon (PL and PB, below right panel) in the lateral side.